

REMARKS

Claims 89-102, 104-106, 108, 124, 125, 127-130, 132-135, 137-147, and 149-164 remain pending after response.

Withdrawal of Election of Species

Applicant acknowledges with thanks the withdrawal of the election of species requirement, as well as the rejoinder of claims 102, 144 and 156 with the previously-examined claims.

Objections to Claims

Claims 97, 100, 137, 138, 150, and 151 stand objected to as depending from a rejected claim, but would be allowable if rewritten in independent form. For the reasons set forth in detail below, all pending claims are believed to be directed to allowable subject matter.

Applicable Legal Standard

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

“There are three possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons of ordinary skill in the art.” *In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998).

“In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before him to make the proposed substitution, combination, or other modification.” *In re Linter*, 458 F.2d 1013, 1016, 173 USPQ 560, 562 (CCPA 1972).

Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. “The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art.” *In re Kotzab*, 217 F.3d 1365, 1370, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). See also *In re Lee*, 277 F.3d 1338, 1342-44, 61 USPQ2d 1430, 1433-34 (Fed. Cir. 2002) (discussing the importance of relying on objective evidence and making specific factual findings with respect to the motivation to combine references); *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

The Supreme Court of the United States has recently held that the teaching, suggestion, motivation test is a valid test for obviousness, but one which cannot be too rigidly applied. See *KSR Int'l Co. v. Teleflex Inc.*, No. 04-1350, slip op. at 11 (U.S. April 30, 2007).

The Supreme Court in *KSR Int'l Co. v. Teleflex, Inc.*, No. 04-1350 (U.S. April 30, 2007) reaffirmed the Graham factors in the determination of obviousness under 35 U.S.C. § 103(a). The four factual inquiries under Graham are:

- (a) determining the scope and contents of the prior art;
- (b) ascertaining the differences between the prior art and the claims in issue;
- (c) resolving the level of ordinary skill in the pertinent art; and
- (d) evaluating evidence of secondary consideration.

Graham v. John Deere, 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966).

The Court in *KSR Int'l Co. v. Teleflex, Inc.*, *supra.*, did not totally reject the use of "teaching, suggestion, or motivation" as a factor in the obviousness analysis. Rather, the Court recognized that a showing of "teaching, suggestion, or motivation" to combine the prior art to meet the claimed subject matter could provide a helpful insight in determining whether the claimed subject matter is obvious under 35 U.S.C. § 103(a).

Even so, the Court in *KSR Int'l Co. v. Teleflex, Inc.*, *ibid.*, rejected a rigid application of the "teaching, suggestion, or motivation" (TSM) test, which required a showing of some teaching, suggestion, or motivation in the prior art that would lead one of ordinary skill in the art to combine the prior art elements in the manner claimed in the application or patent before holding the claimed subject matter to be obvious.

Applicant submits that the Examiner fails to present a *prima facie* case of obviousness in relation to the cited prior art.

Rejection under 35 USC 103(a)

Claims 89-96, 98, 99, 101, 102, 104-106, 108, 124, 125, 127-130, 132-135, 139-147, 149 and 152-164 stand rejected under 35 USC 103(a) as being unpatentable over *Jackson* in view of *Riley et al*, *Klampfer et al*, *Wawretschek et al*, *Herschler '421*, *Herschler '039* and *Menon et al*. This rejection is respectfully traversed.

The claimed method is neither disclosed nor suggested by the cited prior art, as is apparent from the following discussion of the deficiencies of the prior art relied upon by the Examiner.

By way of review, the claims as presently on file are all directed to a method of treating neoplastic disease in a human or animal patient comprising administering to the patient an anti-neoplastic effective amount of a composition comprising:

- (a) a physiologically acceptable source of assimilable copper;
- (b) salicylic acid or an alkali or alkaline earth metal salt thereof;
- (c) vitamin C; and
- (d) a physiologically acceptable source of assimilable manganese.

These claims are based on the inventor's surprising discovery that a composition containing at least these four components (and optionally, but not necessarily, others) is effective in combating neoplastic disease. The data provided in the application (cf. Examples 11 to 18) clearly demonstrates the striking effects of compositions comprising said four components in treating cancer. In each of said studies, the compositions in accordance with the invention (consisting of sodium salicylate, vitamin C, manganese orotate, and copper gluconate or orotate) were effective in inhibiting, halting, or even reversing tumour growth. Reference is made, in particular to Examples 11 and 13 and related Tables 1 and 2 of the application, in which studies

the mice treated with the claimed compositions had significant reductions in tumour mass and increases in life expectancy as compared to the control mice.

As further evidence of the efficacy of the claimed compositions in treating neoplastic disease, applicant submits herewith reports of two further animal studies investigating the anti-neoplastic effects of a composition (referred to as CV247) in accordance with the invention (consisting of sodium salicylate, vitamin C, manganese gluconate, and copper gluconate). Again this data demonstrates that animals treated with the compositions of the present invention show a clear reduction in tumour mass/volume as compared to the control animals untreated with the compositions. Moreover, this data also supports the teaching of the present application that it is the claimed combination of components that leads to the particular efficacy of the claimed compositions in treating cancer. In particular, the compositions comprising all the claimed components (CV247) had demonstrably greater benefits than sodium salicylate on its own or in combination with vitamin C.

The applicant maintains that the presently claimed methods are neither disclosed nor suggested by said art, for the following reasons.

Jackson

Jackson discloses a dietary supplement for supplementing the micronutrient and phytochemical needs of a woman at various stages during her life cycle to prevent or reduce the risk of (not treat) a number of conditions, including some cancers. The supplement is a composition comprising copper, vitamin C, manganese and twelve other components (including iron and zinc) in admixture with a biologically acceptable carrier (see column 2, line 34 to column 3, line 21).

The purpose of the compositions disclosed in Jackson is to supplement the specific micronutrient and phytochemical needs of a woman during each of her adult life stages, with the objective of generally promoting her well being and thereby hopefully preventing or reducing various health risks to which she may, during this period, be exposed (see column 1, lines 4 to 9). Amongst the listed health risks are “some cancers” (see column 1, lines 21 to 28). It is disclosed (see column 1, lines 26 to 28) that the incidence and risk of these conditions varies with each life stage and has been shown to be influenced by diet and dietary supplements.

There is, however, no suggestion in Jackson that the dietary supplements disclosed therein would be of any utility where *treatment of cancer* is concerned. As the skilled person would readily appreciate, while a dietary supplement may be of benefit in maintaining the general well being and health of an individual, and thus conversely have some utility in reducing the chances of that individual contracting cancer or some other form of disease or illness, this by no means raises any expectation that the supplement would be effective in combating cancer or other diseases once established.

Equally, the compositions disclosed in Jackson do not contain salicylic acid, or any alkali or alkaline earth metal salt thereof. Thus, there is no suggestion in Jackson that salicylic acid, or any alkali or alkaline earth metal salt thereof, would be useful even in reducing the risk of cancer being contracted, let alone that it would be of any use in treating cancer.

Riley

Riley discloses (see column 1, lines 20 to 26) a modular system of multivitamin and mineral supplementation to replace micronutrients lost as a result of lifestyle factors and inadequate diet thereby improving public health by insuring adequate intake of micronutrients needed for disease prevention. Modules 4, 5 and 6 of Riley contain aspirin, with Modules 5 and

6 further including copper, vitamin C, and manganese, together with 23 other components including iron and zinc (see Table II). It is furthermore stated (see column 6, line 62 to column 7, line 6) that the modular system can be used to reduce the risk of chronic diseases such as cancer (amongst others).

Again however, and as with Jackson, there is in Riley no suggestion in that the disclosed modular systems would be of any benefit in treating cancer.

Equally, whereas the claims of the present application require the use of salicylic acid or an alkali or alkaline earth metal salt thereof, Modules 4, 5 and 6 of Riley use aspirin, i.e. acetylsalicylic acid, which is not within the scope of the claims. Aspirin is used in Riley primarily for its antiplatelet aggregating capacity, so as to reduce the risk of coronary heart disease (see column 5, lines 9 to 13 and 31 to 39), although brief reference is made to it also being able to *reduce the risk of* certain cancers (column 16, lines 24 to 26). Moreover, as evidenced by the extracts from Martindale “The complete Drug Reference”, 32nd edition (1999) (of record), this antiplatelet aggregating capability only occurs with *acetylated salicylates*, i.e. it is present for aspirin but not for the claimed salicylic acid or salicylates.

While referring to aspirin per se in Table II, Riley does also state at certain other points that “aspirin or the like” may be used (see column 5, lines 9 to 14, column 16, lines 15 to 17, and column 21, lines 48 to 62). However, what is meant by this is further explained at column 21, lines 48 to 62. As explained in this passage, according to Riley either aspirin or a “bioequivalent thereof” should be used, or a compound used “which can be easily converted to aspirin”, it being confirmed at lines 59 to 62 that the compound to be used should be “extracted, processed, tested, and utilised either as is (bioequivalent) or converted to acetylsalicylic acid (aspirin)”.

As explained above, salicylic acid and alkali or alkaline earth metal salts thereof *cannot*, in the context of Riley, be considered bioequivalents of aspirin, it being common knowledge (see Martindale) that they lack the very anti-platelet aggregating capability for which aspirin is, in Riley, primarily used. Thus, there is in fact no suggestion in Riley to use salicylic acid, or any alkali or alkaline earth metal salt thereof, in the modular systems described therein, only that salicylic acid and salicylates may be a suitable source of acetylsalicylate (aspirin).

It follows, therefore, that, like Jackson, Riley also does not disclose the use of salicylic acid, or any alkali or alkaline earth metal salt thereof even in a composition for reducing the risk of cancer, let alone as part of a composition for treating cancer.

Klamfer

Klamfer teaches that sodium salicylate (Na-Sal) can induce apoptosis in several myeloid leukaemia cell lines tested. It also teaches that, at sub-lethal concentrations, Na-Sal potentiates the known apoptotic effects of growth factor withdrawal or treatment with daunorubicin. No in vivo data is presented, but on the basis of the aforementioned in vitro data the authors propose that Na-Sal *may* have therapeutic *potential* for the treatment of human leukaemia. The document also refers to the previous papers investigating the use nonsteroidal anti-inflammatory agents (NSAIA) in general as chemopreventative agents (i.e. as agents for *preventing*, rather than *treating*, cancer). There is no teaching in this document regarding vitamin C, manganese, or copper, either on their own or in combination with sodium salicylate. In particular, there is no disclosure of the use of these combination in combination as a treatment for cancer, nor is there any teaching as to what effect such a combination might have on the effects of sodium salicylate, whether in terms of inducing cell apoptosis, treatment of cancer, or otherwise.

Wawretschek

Wawretschek discloses a means of reinforcing the pharmacological action of medicaments which exhibit an affinity for linking with blood proteins *in-vivo* and *in-vitro*. It is an object of the invention to find a means which is capable of providing a controlled increase of that portion of the drug to be used which is not bonded to the serum albumen (see column 1, lines 55/58) and that this is achieved by the use of orotic acid and/or a physiologically tolerable orotic acid salt.

In Example 5 of Wawretschek the analgesic efficacy of sodium salicylate is examined both alone and in a binary composition with choline orotate. There is no disclosure of the use of copper, manganese, or vitamin C (or, for that matter, iron, sulphur or zinc). Moreover, there is no teaching whatsoever relating to the treatment neoplastic disease.

DE 2457424

DE 2457424 teaches that zinc orotate is effective against cancer. There is no teaching regarding *any of* sodium salicylate, vitamin C, copper, or manganese – in other words, there is no teaching regarding any of the compounds required by any of the independent claims of the present application.

Herschler ('421)

Herschler '421 discloses (see column 1, lines 12 to 18) the use of methylsulphonylmethane ("MSM") to ameliorate the symptoms of stress (specifically gastrointestinal upset, inflammation of the mucous membranes and allergic reactions). In one example (Example VIII, column 12, lines 11 to 47), MSM is administered with and without ascorbic acid (Vitamin C) to treat mucous membrane inflammation at least partly associated with

lung tumours. Treatment (both with and without Vitamin C) appears to have alleviated inflammation, and caused significant regression of tumour mass.

Thus, Herschler teaches the use of compositions comprising MSM, optionally including Vitamin C, for the treatment of lung tumours and associated inflammation of the mucosa.

However, there is no disclosure of the use of copper, manganese, or salicylic acid or an alkali or alkaline earth metal salt thereof, as part of a treatment for neoplastic disease.

Herschler ('039)

Herschler '039 discloses (see abstract) that MSM is an assimilable form of sulphur. It also discloses (see Example 36) that supplementation of diet with 2 wt % MSM can inhibit DMBA-induced mammary carcinoma in rats and (see Example 37) that supplementation of diet with 3 wt % MSM in water can protect against otherwise lethal spontaneous mouse lymphomas. As with Herschler '421, however, there is no disclosure of the use of copper, manganese, or salicylic acid or an alkali or alkaline earth metal salt thereof, as part of a treatment for neoplastic disease.

Memnon

Memnon teaches (according to the abstract thereof cited by the Patent Office), that vitamin C was effective in *in-vitro* studies in reducing cell viability of two prostate cancer human cell lines. On this basis, the authors suggest that vitamin C is an anti-cancer agent for prostate cancer cells. The document also makes reference to previous studies describing a “protective role” of vitamin C in certain types of cancer (i.e. presumably relating to the use of vitamin C in preventing contracting cancer, rather than in cancer treatment).

There is no teaching in this document regarding sodium salicylate, manganese, or copper, either on their own or in combination with vitamin C. In particular, there is no disclosure of the

use of these combination *in combination* as a treatment for cancer, nor is there any teaching as to what effect such a combination might have on the effects of vitamin C in terms of cancer treatment or otherwise.

Thus, to summarise, the combined teachings of the above cited prior art amount to no more than the following:

- that vitamin and mineral supplements can be used to improve general health (which may have a knock on effect in reducing the risk of contracting diseases in general);
- that aspirin (or similar acetylated salicylates) if added to vitamin supplements may be beneficial in terms of general health and reducing the risk of contracting disease;
- that sodium salicylate has, on its own or in combination with the removal of growth factor or addition of daunorubicin, been shown to be effective *in-vitro* as in inducing apoptosis in certain cell lines and, for that reason, it has been proposed that sodium salicylate *may* have therapeutic *potential* as an anti-cancer agent;
- that sodium salicylate is a general analgesic whose analgesic effect can be improved by addition orotic acid or a tolerable orotate salt;
- that MSM *may* have some efficacy in treating neoplastic disease; and
- that vitamin C has been shown to be effective *in-vitro* in reducing cell viability in certain cell lines and has for that reason been suggested as an anti-cancer agent for cancer cells.

These combined teachings, as outlined above, fall far short of disclosing or suggesting a method as presently claimed. Much of the art cited *does not refer to cancer treatment at all*, while such art which does refer to cancer treatment refers only to the possible potential use of certain compounds in singly and isolation (or else in combination with compounds other than those claimed). There is no teaching in the art as a whole regarding a combination of manganese, copper, vitamin C and sodium salicylate (or salicylic acid or other alkali or alkaline earth metal salts thereof) as a treatment for cancer, nor is there any obvious reason why one of ordinary skill in the art, on reading the above cited documents (not to mention the multitude of other prior art documents relating to possible cancer treatments) would have suddenly decided to investigate or adopt such a combination as a treatment for cancer.

In particular, the cited art provides no indication or suggestion of the additive benefits of the claimed combination of components in treating cancer, or of the resulting efficacy of the combination as a cancer treatment, absent which the claimed combination is but one of hundreds if not thousands of combinations which merely could but in theory have been investigated.

For example, based on the teaching of the newly cited Klamfer, one skilled in the art might have been motivated to investigate further the use of sodium salicylate, on its own or in combination with daunorubicin, as a treatment for cancer. Alternatively, based on the newly cited Memnon, one skilled in the art might have been motivated to investigate further the use of vitamin C on its own.

However, there is no reason why it would have been obvious to one of ordinary skill in the art to investigate a combination of sodium salicylate and vitamin C as a treatment for cancer, let alone to investigate the further combination of these compounds with copper and manganese (which are not suggested in the cited art as having any effect on cancer treatment).

There are many putative and established anti-cancer agents available, but that does not make the combination of any and all such agents obvious. There is no reason to consider, in advance, that two different anti-cancer compounds operating (according to Klamfer and Memnon) in different manners (as regards their biochemical targets) will have beneficial effects if combined – as is illustrated by Klamfer itself, which considers the synergistic effects of sodium salicylate and another known anti cancer agent (daunorubicin) surprising. Indeed, it is just as possible that two anti-cancer agents could provide no additive effect, or that the two agents could have conflicting effects (thus reducing overall efficacy).

Likewise, there is even less reason to consider that adding a compound having *no known anti-cancer effect* would be beneficial, whereas adding such additional compounds could still have adverse effects on the efficacy of the composition, or other unforeseen and undesired side effects. Thus where, as in the present case, the cited art provides no teaching to suggest to one of ordinary skill in the art that a claimed combination of compounds could or should beneficially be used in combined in a claimed method of therapy, it is submitted that the claimed subject matter is not obvious.

While acknowledging that the claimed invention is distinguished in that the art does not expressly disclose the claimed methods, the Examiner nonetheless contends (paragraph bridging pages 3 and 4) that:

“ the prior art amply suggests the same...as such, it would have been well within the skill of and one of ordinary skill in the art would have been motivated to modify the prior art by providing the copper, iron, zinc and manganese as salts of orotate so as to increase the efficacy of the sodium salicylate and to combine copper, iron, zinc and manganese with sodium salicylate and vitamin C with the expectation that the composition would be suitable for use in pregnant women and for treatment of cancer, to further add MSM as it is effective in treating cancer.”

It is respectfully submitted that, for the reasons outlined above, in no way does the prior art "amply suggest" the claimed invention, it would not have been "well within" the ability of one of ordinary skill in the art to arrive at the claimed invention, nor would one of ordinary skill in the art "have been motivated to modify the prior art" so as to arrive at a method as claimed. Much of the prior art cited does not relate to treating neoplastic disease, and it would not have been obvious to one of ordinary skill in the art to refer to such documents at all, nor would one of ordinary skill have obtained any useful information regarding cancer treatment had he or she done so. Conversely, the art which does refer to cancer treatment teaches only to the possible potential use of certain of the claimed compounds in isolation (or in combination with compounds other than those claimed), and provides no teaching that would have lead one of ordinary skill in the art to the presently claimed combination as a treatment for cancer.

At page 4, last two paragraphs, the Examiner notes that:

"one cannot show non-obviousness by attacking references individually where the rejections are based on combinations of references...further, the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference... nor is it that the claimed invention must be expressly suggested in any one or all of the references... motivation is not a required element of the prima facie case of obviousness."

The applicant's arguments are not based on any of the above cited rationales. Rather, for reasons set out above, the applicant simply contends that the requirements for rejecting the application under 35 U.S.C. 103(a) have not been met, i.e., that the combined teachings of the references would not have suggested the claimed invention to one of ordinary skill in the art.

As regards motivation, the applicant's arguments that the art provides no motivation to arrive at the claimed invention are in response to the Examiner's assertion that the art provides such a motivation (as noted above).

Finally, on page 5, the Examiner argues that:

"The mere fact that one or more of the references do not disclose treatment of cancer or all of the components in a single reference does not make the claimed invention non-obvious. Although the claims require that the composition have an anti-neoplastic effect, there is no requirement that all the components have an anti-neoplastic effect so long as the composition has an anti-neoplastic effect. Since the prior art discloses and/or suggests that ascorbic acid, zinc, sodium salicylate and methylsulfonylmethane has anti-neoplastic effects, one of ordinary skill in the art would expect that a combination of the same with minerals such as copper, manganese and iron, including salts of the same with orotate as the anion, would have anti-neoplastic effects. Since the Applicant has not provided evidence of the criticality of each element in the combination of pharmacologically active components, it would have been well within the skill of one of ordinary skill in the art to add copper, iron and manganese to the combination of ascorbic acid, zinc, sodium salicylate and methylsulfonylmethane, with the expectation that the combination without other pharmaceutically active components would be effective as anti-neoplastic composition and that the copper, iron and manganese would provide nutrients to the cancer patient. Further, since the prior art recognises that the zinc in the zinc orotate is the active component, it would have been well within the skill of one of ordinary skill in the art to use other pharmaceutically acceptable anions as desired."

Again, as outlined above, it is not the applicant's contention that the claimed invention is non-obvious because the claimed combination is not disclosed in a single reference, but rather that is not suggested or rendered obvious from the art as a whole. While there is no requirement that all the components have an anti-neoplastic effect so long as the composition has an anti-neoplastic effect, the relevant question is not whether the claimed components each have an anti-neoplastic effect, *but whether it would have been obvious to one of ordinary skill in the art to administer the combined combination of components as an anti-neoplastic treatment.*

As explained above, it would not have been obvious from the art cited that even the combination of ascorbic acid and sodium salicylate (suggested individually in Klamfer and Memnon as compounds having possible utility as anti-cancer agents) would be beneficial (as compared to their use individually), let alone that other compounds having no indicated anti-neoplastic effects (copper and manganese) could or should beneficially be added.

The fact that copper and manganese are used in multivitamin compositions does not make it obvious to add them to an anti-cancer composition (or else multivitamins would be added to all anti-cancer compositions, which is clearly not the case). It is an inevitable fact that a pharmaceutical active may interact unfavourably with another active resulting in loss of efficacy or adverse side effects in one or more patient group (reference being made to any standard pharmacopoeia which, under any drug entry, will normally have sub-headings on known interactions with other actives). Thus, the standard practice when making a pharmaceutical composition is to incorporate only those components necessary to treat the condition for which it is to be administered. This minimises the risk of unforeseen side effects resulting from interaction between multiple active components, either in the population at large or in patient groups at high risk (such as pregnant women, diabetics, immunologically compromised individuals, children, and so forth). It is then left to the physician treating a patient, on diagnosing a particular patient's needs (which may or may not involve a need for both cancer treatment and dietary supplements), to determine what *separate* compositions (for treating the *separate* conditions afflicting the individual patient) can be safely and effectively prescribed or administered.

Moreover, the applicant has now provided evidence, as submitted herewith, demonstrating the enhanced effects in treating cancer of the claimed combination of compounds,

as compared to the components individually. Such enhanced effects of the claimed combination are not obvious from the cited art nor, for the reasons set out above, would it have been "well within the skill of one of ordinary skill in the art" to arrive at the presently claimed combination of components in the expectation that the resulting combination would be effective as anti-neoplastic composition.

The rejection under 35 USC 103(a) is this without basis and should be withdrawn.

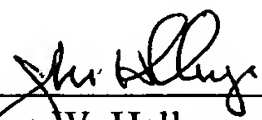
The application is now in condition for allowance. Allowance of claims directed to the generic invention is believed proper.

Payment in the amount of \$525.00 is submitted herewith as payment for the requested three month extension of time.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 
James W. Hellwege, #28,808
P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

JWH
3920-0110P

Attachments: (1) The results of a preliminary study to test the effect of CV247 on the rate of growth of implanted RMA lymphoma cells in C57B1/6 mice
(2) Study to investigate the anti-cancer potential of CV247 and its constituents dosed to C57BL mice bearing a synergistic tumor



The results of a preliminary study to test the effect of CV247 on the rate of growth of implanted RMA lymphoma cells in C57B1/6 mice.

The objective of the study was to undertake a controlled trial of CV247 to evaluate its effects on the growth of transplantable tumours in inbred mice.

A total of 50 male C57B1/6 mice were each injected subcutaneously with 3×10^6 RMA lymphoma cells, a dose known to give 100% tumour "take". 24 experimental mice were treated with 0.1 ml of CV 247, which contained:

Mn gluconate 0.2mg (0.025mg Mn)
 Cu gluconate 0.2mg (0.028mg Cu)
 Vitamin C 4.0mg
 Na salicylate 3.5mg

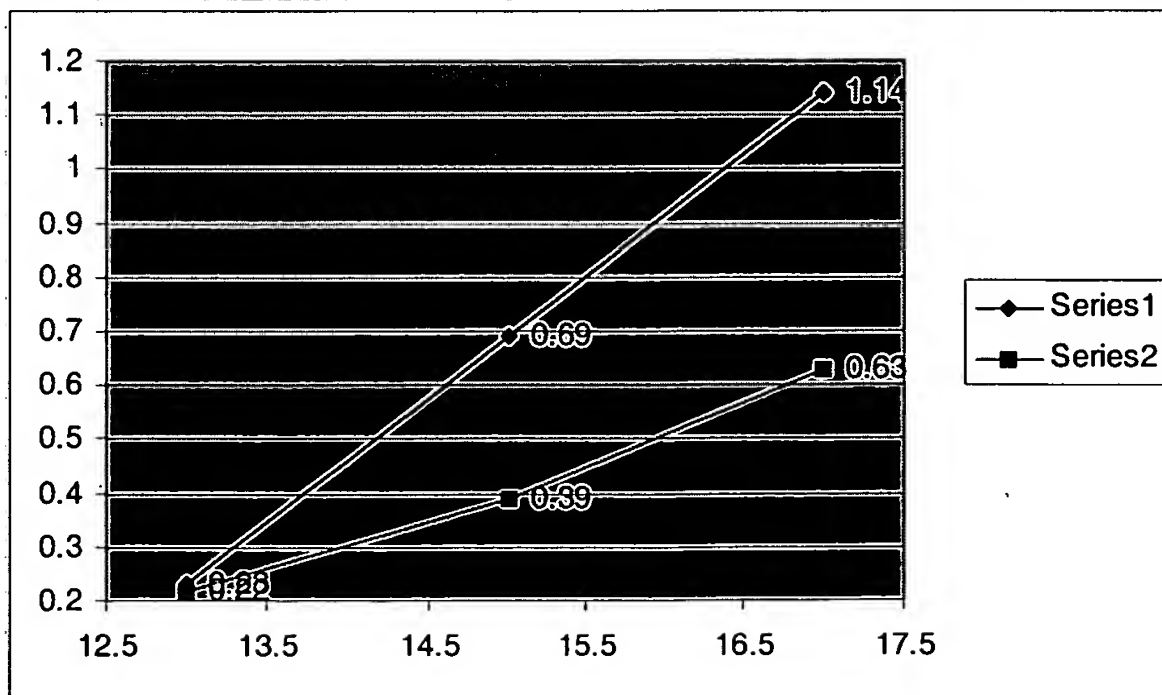
There was no significant difference between experimental and control animals during the early time points, but there was a statistically significant difference in the size of the tumours measured from day 17, and also for the weight of the excised tumours. In addition, at day 17, 4 tumours were too small to measure in the animals treated with CV247, compared with only 1 in the control group. In a number of mice more than 1 tumour grew along the injection needle tract, but this was considerably more frequent in the control group (10 mice) compared with the experimental group (1 mouse). In 3 control mice, tumours could not be excised because they were infiltrating deeper tissues.

There were no side effects attributable to CV247.

At the doses used in this controlled study, CV247 demonstrated a convincing measurable effect on the growth rate of RMA thymoma in mice.

TABULATED MEAN DATA

Group	Size day 13	Size day 15	Size day 17	Weight day 17
Exp (series 2)	0.22	0.39	0.63	0.46
Con (series 1)	0.23	0.69	1.14	0.92



Study to investigate the anti-cancer potential of CV 247 and its constituents dosed to C57BL mice bearing a syngeneic tumor

Summary

This study assessed the anti-tumour activity of CV247 and combinations of the formulation's constituents against LL2/LLc1 tumours in C57BL mice. Lewis Lung Carcinoma (LLc). The effect of the agents was compared to untreated control animals bearing tumours. Gemcitabine, a highly active anti-tumour agent, was used as a positive control.

Lewis Lung Carcinoma (LLc) is a highly metastatic and drug resistant murine non-small cell lung (NSCL), and has been widely used to study tumour growth, metastasis and chemotherapy. Gemcitabine alone and in combination has been shown in previous studies to be efficacious against LLc grafts, and so was deemed a suitable positive control in this experiment.

LL2/LLc1 cells were injected subcutaneously into female C57BL6/J mice. A total of 110 mice were allocated to 10 dose groups. A first group assessed a dosing regimen of CV247 administered at 10mL/kg daily from the day of tumour inoculation, for a period of 21 days. All other regimens commenced 7 days after inoculation. In second, third and fourth groups, CV247 was dosed daily at 3mL/kg, 10mL/kg and 20mL/kg, respectively, for 14 days. Groups five to eight looked at the components of CV247, Sodium Salicylate (35mg/kg), Sodium Salicylate + Ascorbic Acid (35mg/kg+40mg/kg), Sodium Salicylate + Ascorbic Acid + Copper Gluconate (35mg/kg+40mg/kg+2mg/kg), or Sodium Salicylate + Ascorbic Acid + Manganese Gluconate (35mg/kg+40mg/kg+2mg/kg), being dosed once daily for 14 days. In group 9 Gemcitabine was administered every 3rd day on 5 occasions commencing 1 week after tumour inoculation. Group 10 was left untreated. CV247 and its components were administered via oral gavage. Gemcitabine was administered via intraperitoneal injection.

The CV247 composition formulation as administered consisted of:

Sodium Salicylate	3.5mg/mL
Ascorbic Acid	4mg/mL
Copper Gluconate	0.2mg/mL
Manganese Gluconate	0.2mg/mL

21 days after tumour inoculation all animals were sacrificed. Tumours were excised and weighed and evaluated macroscopically. In addition, the tumours from the CV247 (10mL/kg), SS+AA, SS+AA+MG, Gemcitabine, and untreated control groups were subjected to histological scoring.

Tumour weights (see Figure below) showed a significant difference between CV247 and the untreated control.

Macroscopic examination of tumours on excision revealed differences in tumour structure between treatment groups. Tumours treated with CV247 and SS + AA + MG appeared to be fluid filled and spongy in comparison to the untreated controls and other component groups. Gemcitabine treated tumours were slightly smaller and did not appear to contain much fluid.

For the histological scoring, tumour tissue (formalin fixed) from each animal was bisected, processed, embedded and sectioned for haematoxylin and eosin staining.

Each slide was examined for the extent of intra-tumoural necrosis, and a score assigned as follows:

- 0 No necrotic foci
- 1 <25% of the area of the tumour is necrotic
- 2 25-50% of the area of the tumour is necrotic
- 3 >50% of the area of the tumour is necrotic

The resulting mean group scores for intra-tumoural necrosis were:

Untreated	1.7
Gem	1.11
SS+AA+MG	1.25
SS+AA	1.37
CV247	1.2

This study demonstrated potential for CV247 to be an anti-tumour agent. The decrease in final tumour weight indicates a mechanism for tumour reduction that is not necessarily related to tumour volume. Speculatively it may be suggested that an immunological process is initiated that results in break down the tumour core, therefore reducing the weight (but not size) of LL2/LLc1 carcinomas.

CAM/13 Phase 3 Mean Tumour Weights

